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Single-molecule Immunofluorescence Tissue Staining Protocol for Oligomer Imaging V.2

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Protocol status: Working
We use this protocol and it's working

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Abstract

This protocol details background fluorescence quenching and immunofluorescence staining of human brain tissue for oligomer imaging.

Attachments

kb3ib25np.pdf
154KB

Guidelines

- Use only clean bottles, flasks, magnetic stirrers, tweezers, weighing spatulas, measuring cylinders – everything should be cleaned, dried and covered if left on the side before next use.
- Everything should be handled with clean tweezers – gloves should not touch the samples, solutions and ideally anything placed into the solutions where the slides are.

Materials

Materials and Reagents

- Microtome
- Glass slides
- Xylene solution
- 100% alcohol
- Methanol
- Hydrogen peroxide (H_20_2) solution
- Citrate buffer pH6
- Milli Q water
- Pressure cooker
- PBS
- Goat Serum 10%
- AlexaFluor antibody
- 0.1% Sudan black solution
- Vectashield
- Overslip

Troubleshooting

Immunofluorescences staining protocol for oligomer imaging

10m

1 Cut 8 µm tissue sections on a microtome and load onto glass slides.

2 Dry slides Overnight at 37 °C – **cover over the top**. 10m

3 Before staining commences keep slides for a few hours but ideally Overnight at 60 °C . 10m

4 De-wax sections through three pots of xylene solution. Use each fresh pots of xylene each time.

4.1 De-wax section through pot of xylene solution for 00:02:00 . (1/3) 2m

4.2 De-wax section through pot of xylene solution for 00:02:00 . (/23) 2m

4.3 De-wax section through pot of xylene solution for 00:02:00 . (3/3) 2m

5 Take sections through two pots of 100% alcohol.

> Note
>
> Use fresh pots each time – methylated spirits.

5.1 Take sections through pot of 100% alcohol for 00:02:00 . (1/2) 2m

5.2 Take sections through pot of 100% alcohol for 00:02:00 . (2/2) 2m

6 Put slides into methanol + hydrogen peroxide (H_2O_2) solution (100 ml: 1 ml) for 00:10:00 at Room temperature **fresh pot each time**. 10m

Note

This process will block any staining of endogenous peroxidase in the tissue sections.

7 Perform necessary antigen retrieval pretreatments by pressure cooking in citrate buffer.

7.1 Pressure cook sections in citrate buffer pH 6 for 00:10:00 at pressure (wait for it to release high pressure air) in **cleaned pressure cooker**. 10m

7.2 Cool under running **Milli Q** water – *never* under tap water.

8 Block non-specific antigen/antibody binding by placing sections in **PBS and Goat Serum 10%** (**taken from the DNA PAINT protocol**) for 00:30:00 . 30m

9 Apply primary antibody for 01:00:00 at Room temperature . 1h

10 Wash in PBS with fresh buffer (**at least filtered if not cell culture grade**).

10.1 Wash 00:05:00 in PBS clean squirty bottle with fresh buffer. (1/3) 5m

10.2 Wash 00:05:00 in PBS clean squirty bottle with fresh buffer. (23) 5m

10.3 Wash 00:05:00 in PBS clean squirty bottle with fresh buffer. (3/3) 5m

11 Apply secondary AlexaFluor antibody for 01:00:00 at Room temperature in the dark. 1h

12 Wash in PBS.

12.1 Wash 00:05:00 in PBS in the dark. (1/3) 5m

12.2 Wash 00:05:00 in PBS in the dark. (2/3) 5m

12.3 Wash 00:05:00 in PBS in the dark. (3/3) 5m

13 Add **filtered (0.22 um)** 0.1% Sudan black solution (0.1% sudan black/70% ethanol) for 00:10:00 at Room temperature in the dark. 10m

14 Wash 2-3 times in 30% ethanol.

15 Mount section with Vectashield and coverslip (**Plasma cleaned slides**).

16 Take for imaging.